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# **The impact of temperature on the transformation of illicit drug biomarkers in wastewater**

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16   **Abstract**

17   In the emerging field of wastewater-based epidemiology (WBE), temperature – a well-known, key factor,  
18   influencing the transformation kinetics of organic chemicals, has thus far been ignored in predicting  
19   chemical consumption rates in urban catchments. This is problematic as WBE data are collected from  
20   and compare sewer catchments with highly varying wastewater temperatures.

21   In this study, we assessed, for the first time, the influence of temperature on the transformation of  
22   biomarker transformation in wastewater and its ensuing implications on the back-calculation of chemical  
23   consumption rate in urban catchments using the example of selected illicit drugs. Literature data, obtained  
24   in laboratory-scale experiments, on the stability of drug biomarkers in untreated wastewater – occurring  
25   at trace levels – was systematically reviewed, and transformation rates obtained at different temperatures  
26   were collected. Robust correlations, using the Arrhenius equation, were inferred to describe the  
27   transformation of selected cocaine and morphine biomarkers in environmentally relevant temperature  
28   ranges (from 2–9°C to 30–31°C), with estimated  $\theta$  coefficients between 1.04 and 1.18. These  
29   empirically-derived relationships were used to assess the influence of temperature on the transformation  
30   of drug biomarkers during in-sewer transport and its effect on the back-calculation of drug consumption  
31   rate in synthetic urban catchment scenario simulations. As for quantifying the uncertainty of temperature  
32   effects, up to 4-fold increase in removal efficiency was estimated when wastewater temperature increased  
33   from 15 °C to 25°C – a range representative, notably, to seasonal variations in continental urban  
34   catchments, e.g., Spain, where some of the highest drug consumption rates are observed in Europe.  
35   Findings from this study can help reducing the uncertainty intrinsic to wastewater-based epidemiology  
36   studies, and will be beneficial in comparing chemical consumption estimates from different catchments  
37   worldwide.

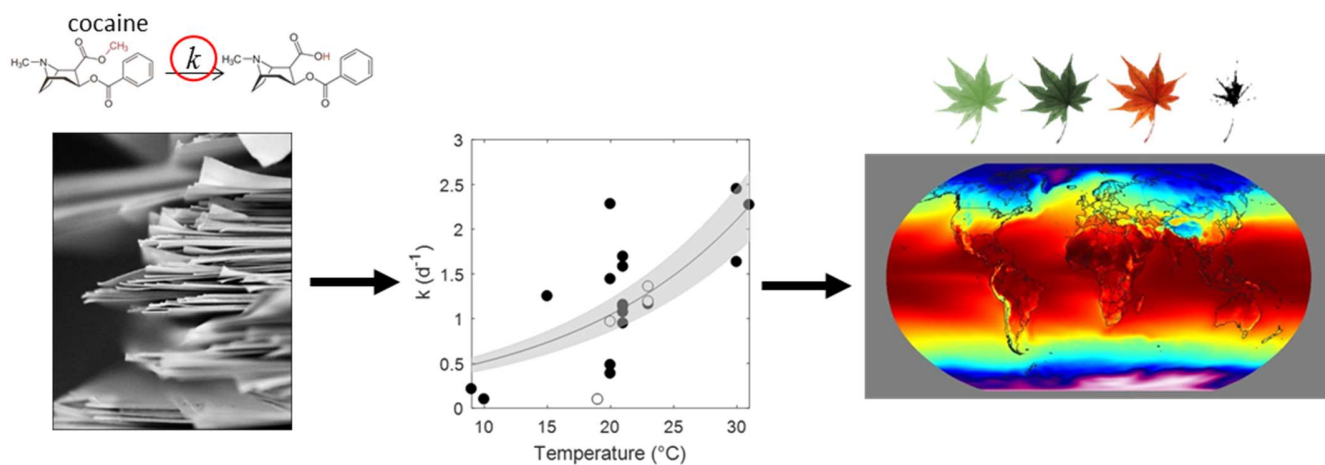
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39 **Keywords:** Wastewater-based epidemiology, stability, temperature, biotransformation, Arrhenius  
40 equation, illicit drugs  
41

## Highlights:

- Assessing impacts of temperature on biomarker transformation and on WBE predictions.
- Broad literature review on transformation rates combined with temperature data
- Robust, Arrhenius-based correlation identified to account for temperature effects; described temperature-dependent transformation of biomarkers
- Findings facilitate significant reduction of uncertainties in WBE assessment. comparative estimation of drug consumption in WBE studies

## Graphical abstract



## 1. Introduction

Wastewater-based epidemiology (WBE) is a growing research field to improve social behavior predictions in an epidemiological context. It is based on the analysis of substance residues (biomarkers) in wastewater and back-calculation of population consumption/exposure at catchment level. Substance use biomarkers, such as illicit drug abuse and exposure to pesticides, have been the main focus of WBE studies (Gracia-Lor et al., 2017). A number of uncertainties (e.g., chemical analysis, determination of catchment population) have been associated to the determination of community drug use (Castiglioni et al., 2013). Furthermore, neglecting in-sewer transformation can also be a significant source of bias since biomarker concentration levels at the excretion point can differ from the sampling point (Li et al., 2018). In-sewer stability of drugs is mainly associated to abiotic transformation (without the presence of biomass) and biotransformation in the presence of suspended and attached biomass. In WBE studies, two main approaches have been used to translate measured concentration to consumption rate: (i) lumped correction factors that include e.g., excretion ratios and in-sewer transformation; (ii) in-sewer process kinetic models together with excretion ratios. The first approach is commonly used due to its simplicity, with the major drawback of lacking catchment specificity. Conversely, process models explicitly rely on first- or second-order equations (McCall et al., 2016; Plósz et al., 2013; Ramin et al., 2016) to describe transformation kinetics, therefore allowing to account for a number of influencing factors (e.g. redox conditions, in-sewer residence time, transformation pathways and biomarker concentrations) depending on the complexity level. A factor known to influence microbial activity—hence biomarker stability—is temperature. The impact of temperature on the transformation of organic micropollutants has been assessed in activated sludge (Li et al., 2005) and in anaerobic digestion (Carballa et al., 2007). As to illicit drug biomarkers, stability studies in untreated wastewater (Bisceglia and Lippa, 2014; Devault et al., 2017) have overall revealed enhanced transformation kinetics with

76 increasing temperature. While the effect of temperature on microbial growth kinetics is considered in  
77 models for conventional pollutants (e.g., activated sludge models), very few examples exist on  
78 quantifying the temperature dependence of kinetic model parameters for trace organic chemical  
79 transformation (Li et al., 2005; Wick et al., 2009).

80 In sewers, wastewater temperature exhibits seasonal and geographical variations and may further vary  
81 within the same catchment. During a recent Europe-wide sampling campaign (conducted simultaneously  
82 in 47 cities), the temperature of raw wastewater at sampling points was reported in the range between 7  
83 °C and 28°C (Ort et al., 2014). Consequently, the impact of temperature on the stability of drug  
84 biomarkers in sewers may significantly vary from catchment to catchment, and the associated  
85 uncertainties propagating to the back-calculated consumption rate could be reduced in WBE approaches  
86 using more robust temperature models – the main focal area chosen for this study.

87 Considering existing limitations, the objectives of this study were: (i) to assess the effect of temperature  
88 on in-sewer drug biomarker stability, based on findings from published literature; (ii) Use empirical  
89 equations to describe temperature-dependent transformation kinetics of selected biomarkers in  
90 wastewater under aerobic conditions; (iii) to assess the influence of temperature on the in-sewer removal  
91 of drug biomarkers through synthetic urban catchment simulations.

## 93 2. Materials and methods

### 94 2.1. Literature review and data treatment

95 Published scientific literature was reviewed (last update: 31/03/2018) to select drug biomarker stability  
96 studies in untreated wastewater, i.e. without the influence of biofilm. Further screening for sound and  
97 rigorous literature evidences was performed using the following criteria: (i) stability studies were  
98 performed under aerobic conditions; (ii) biomarker transformation kinetics were explicitly reported or  
99 could be derived (calculated) based on presented results (e.g., concentration profiles in batch  
100 experiments); (iii) estimation of model parameters (see Eq. 1) was associated with good match between  
101 measured and predicted concentration profiles ( $R^2 > 0.7$ ). Ten literature studies were eventually selected  
102 (Table 1), providing relevant information on stability of cocaine (COC), ecgonine-methyl-ester (EME),  
103 cocaethylene (CE), norcocaine (NorCOC) and 6-monoacetylmorphine (6-MAM).

104 The first-order transformation rate coefficient ( $k$ , d<sup>-1</sup>) was used as indicator of biomarker stability in  
105 wastewater. Notably,  $k$  accounts for both abiotic and biotransformation kinetics, given that abiotic control  
106 experiments were absent in most of the selected studies. When  $k$  values were not explicitly reported, they  
107 were estimated by fitting experimental data with a first-order kinetic equation (Eq. 1):

$$108 \quad C(t) = C_0 e^{-kt} \quad (\text{Eq. 1})$$

109 where  $C_0$  and  $C(t)$  are biomarker concentrations at time 0 and at time  $t$ , respectively.

110 In two cases (McCall et al., 2016; Ramin et al., 2016), abiotic and biotransformation kinetics were  
111 separately assessed and quantified by estimating the first-order rate coefficients ( $k_{abio}$ , d<sup>-1</sup>) and pseudo-  
112 first-order rate coefficients ( $k_{bio}$ , L g<sup>-1</sup> d<sup>-1</sup>), respectively. The two kinetic indicators were combined to  
113 obtain  $k$  (Eq. 2):

$$114 \quad k = k_{abio} + k_{bio} X_{TSS} \quad (\text{Eq. 2})$$



115 where  $X_{TSS}$  (g L<sup>-1</sup>) denotes the concentration of total suspended solids (TSS) in the experiments. Data  
116 from concentration profiles were extracted, when necessary, using the software *PlotDigitizer* (name of  
117 manufacturer, Country)

118 For each biomarker, the Arrhenius equation (Eq. 3) was used to describe variations in transformation  
119 rates as a function of temperature:

$$120 \quad k_T = k_{25} \theta^{(T-25)} \quad (\text{Eq. 3})$$

121 where T(°C) denotes the temperature, at which a specific  $k_T$  value was derived,  $k_{25}$  the transformation  
122 rate at 25°C and  $\theta$  (-) the exponential Arrhenius coefficient. Parameters  $\theta$  and  $k_{25}$  were estimated for each  
123 biomarker using particle swarm optimization in MATLAB 2016b. A temperature of 25°C was selected  
124 as reference to improve the identifiability of both estimated parameters, as previously suggested  
125 (Schwaab et al., 2007).

126 <Table 1>

## 127 **2.2. Back-calculation procedure**

128 To back-calculate drug concentration at the release point e.g. after toilet flush (unknown), drug  
129 concentration at the influent of wastewater treatment plant (known) is considered in a hypothetical  
130 catchment. In-sewer transformation was simulated using Eq. 1 and assuming an average residence time  
131 of 4.5 h, corresponding to the average residence time in a recent European monitoring campaign (Ort et  
132 al., 2014).

## 133 **2.3. Parameter estimation and quantification of uncertainties**

134 Bayesian inference, employing prior knowledge in terms of model parameters, is employed to estimate  
135  $\Theta$  parameter values using ???. Additionally, propagation of parameter uncertainty onto the back-  
136 calculation results is quantified. Monte Carlo simulations of in-sewer biomarker transformation were

137 performed using prior parameter sets sampled using Latin Hypercube Sampling (LHS, Reference). To  
138 evaluate the impact of temperature on the removal of the selected drugs, three temperature conditions  
139 were considered, being representative of low ( $T=5^{\circ}\text{C}$ ), medium ( $T=15^{\circ}\text{C}$ ) and high ( $T=25^{\circ}\text{C}$ )  
140 temperature.

141

### 142 3. Results and discussion

#### 143 3.1. Temperature-dependent transformation

144 Considerable data variability in  $k$  rate values found in literature was noticed for most all selected drugs  
145 (Fig. 1), even considering the same temperature (e.g., 6-MAM) as a result of factors such as, differences  
146 in stability test conditions used in literature. Nevertheless, overall increase of  $k$  with increasing  
147 temperature was observed, especially when considering the mean of multiple measurements for each  
148 unique temperature.

149 Additionally, for each biomarker, Fig. 1 presents plots of fitted Arrhenius equations (and associated 95%  
150 confidence intervals (CI), shaded areas). Interestingly, many of the calculated data points (not reported  
151 in the original study) and estimated ones (reported in the original study) fall out of the CI (almost 50%  
152 of all data points). A portion of data points were found to be inside the CI, namely McCall et al. (74%),  
153 Bisceglia and Lipa (67%), Mardal et al. (56%), Devault et al. (37%), Baker and Kasprzyk-Hordern  
154 (33%) and van Nuijs et al. (33%). Low transformation (hence below CI) in Senta et al. (2014) and Chen  
155 et al. (2013) could be due to limited oxygen availability in test setups, resulting in lower microbial activity  
156 (Table 1). Conversely, high transformation observed in Ramin et al. (2016) could have resulted from  
157 high oxygen levels ( $\sim$  saturation) in test reactors, determining significant microbial growth during batch  
158 experiments. Beside oxygen levels, under- or over-estimation of  $k$  ( $\text{d}^{-1}$ ) values can be a consequence of  
159 the limited applicability of first-order kinetics to describe biomarker biotransformation, e.g. due to  
160 significant microbial growth or inhibition of biomass. This could be the case for Thai et al. data points  
161 which are placed both above and below CI. Moreover, partitioning of drug biomarkers to solid phases  
162 (suspended particles, reactor walls) are additional processes that need to be accounted for when  
163 estimating  $k$  ( $\text{d}^{-1}$ ) (Ramin et al., 2016).

164 Besides the previously discussed inherent data variability, this may have resulted from the limited  
165 applicability of first order transformation kinetics e.g. due to significant microbial growth during batch  
166 experiments (Ramin et al., 2016).

167 Estimated parameter values  $k_T(\text{d}^{-1})$  and  $\theta$  for the selected biomarkers are reported in Table 2. It can be  
168 noticed that the estimated relative error was low, below 50%, except for NorCOC (0.78%) and parameter  
169 collinearity was low expect for EME (-0.75). This seems to suggest good parameter identifiability, based  
170 on criteria (error < 50% and collinearity < 0.7) set by (Frutiger et al., 2016). Nevertheless, these  
171 thresholds are subjective and the consideration of 25°C as reference temperature allowed for the  
172 improvement of parameter identifiability (achieving lower correlation).

173 Estimated  $\theta$  coefficient values were between 1.04 and 1.18, in agreement with previously reported  
174 values. That is, for primary metabolic processes (relevant for biomass growth) in sewers, Arrhenius-  
175 based temperature corrections have been suggested, with  $\theta$  values of 1.07 and 1.05 for aerobic water  
176 phase and biofilm processes, respectively (Hvitved-Jacobsen et al., 2013). Henze et al. (2000) also  
177 suggested similar coefficients to describe temperature dependency of biological processes in the  
178 Activated sludge model No. 2 (ASM2). These coefficients are ranging from low ( $\theta = 1.04$ ) for hydrolysis  
179 to high ( $\theta = 1.12$ ) for nitrification. Similar  $\theta$  values were also estimated for 17- $\beta$  estradiol (E2)  
180 transformation by activated sludge, ranging from 1.03 to 1.09 for different biomass concentrations (Li et  
181 al., 2005). Wick et al. (2009) considered temperature-dependent biotransformation for successful  
182 prediction of season-dependent pharmaceutical and illicit drugs removal in WWTPs. The correction  
183 factor,  $\theta$ , for organic micropollutants such as pharmaceuticals was estimated in the range of 1.03–1.09  
184 (Joss et al., 2006). Overall, previous and current findings demonstrate that temperature can have  
185 considerable impact on transformation, the extent of which is compound-dependent.

186

187 *3.2. Influence of temperature on back-calculation of drug use*

188 As expected, higher temperature resulted in higher in-sewer removal, with 40% (6-MAM) to almost 4-  
189 fold (EME) increase of removal efficiency from medium to high temperature. Consistently, 6-MAM and  
190 EME have lowest and highest  $\theta$  values (Table 2).

191 These results indicate that accounting for in-sewer transformation is important especially at elevated  
192 temperatures (above 15°C). Consequently, the temperature dependency of  $k$  should be accounted for  
193 explicitly in steady-state and dynamic model simulations. From this stand point, the Arrhenius equation  
194 can be included in existing modeling frameworks for removal of drug biomarkers in wastewater such as  
195 WATS—ASM-X (Ramin et al., 2016). We note that, in this study, the estimation of in-sewer removal  
196 was performed based on individual biomarkers, and the transformation of biomarkers into/from other  
197 biomarkers was neglected. It is common practice to back-calculate the consumption of COC based on  
198 the concentration of its metabolite benzoylecgonine (BE) and COC itself. It has been found that BE,  
199 beside formation, also under go transformation (McCall et al., 2016; Ramin et al., 2016), although some  
200 studies reported negligible in-sewer BE transformation (Bisceglia and Lippa, 2014; Thai et al., 2014).  
201 Further discussion on back-calculation of illicit drug consumption interested readers are referred to  
202 available literature (Castiglioni et al., 2013; Khan and Nicell, 2011).

203 It is evident that further research is crucial for obtaining new evidence on drug stability at different  
204 temperatures, especially for new psychoactive substances. This is generally relevant for other types of  
205 biomarkers beyond illicit drugs, which wastewater-based epidemiologists have gained increasing interest  
206 in (Gracia-Lor et al., 2017). We encourage authors to report conditions at which stability tests were  
207 performed, similarly to Table 1. This would allow for better comparison and consistency evaluation  
208 among different studies.

209

210 **4. Conclusions**

211 This study presents, for the first time, a comprehensive correlation analysis for the temperature  
212 dependence of transformation kinetics (abiotic and biotic) of biomarkers and quantifies its uncertainty  
213 implications on WBE back-calculation results. Five illicit drug biomarkers (COC, EME, CE, NorCOC,  
214 6-MAM) were used in untreated wastewater under aerobic conditions. Following conclusions are made:

- 215 • Although affected by the considerable variability of measured transformation kinetics, the  
216 Arrhenius equation could capture trends of increasing transformation rates with increasing  
217 temperature within the applicability domain (from 2–9°C to 30–31°C).
- 218 • Arrhenius-based equations were estimated for each biomarker and used for removal predictions  
219 during transport in ideal sewers. Up to almost 4-fold removal efficiency was observed when  
220 temperature was changed from 15 °C to 25°C.
- 221 • These findings have considerable implications for back-calculation of drug consumption based  
222 on the analysis of untreated wastewater influents, especially for multi-catchment studies covering  
223 wide geographical areas. Further research should extend the investigation of temperature effects  
224 to (i) a larger number biomarkers; (ii) anaerobic conditions; and (iii) sewer biofilms.

225

226

227

228

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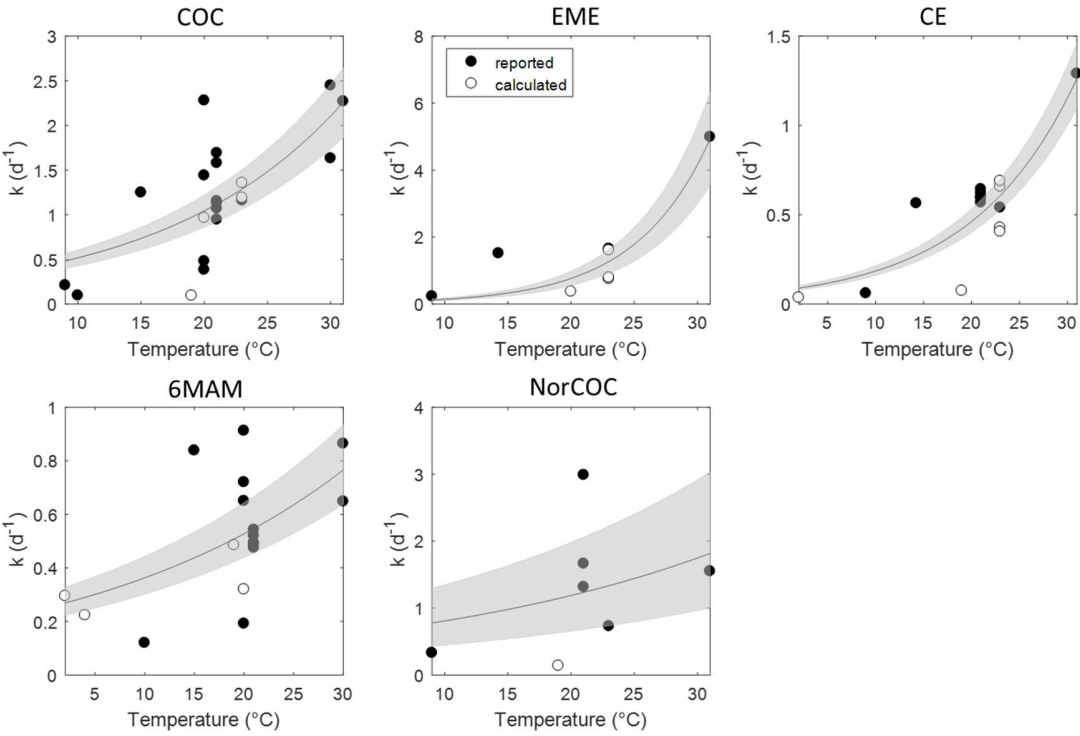
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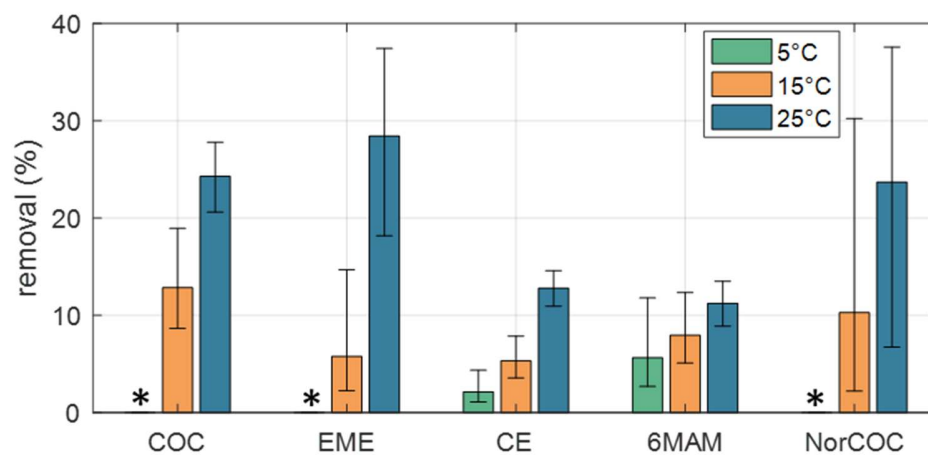
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 314

315 **Figures**



316

317 **Figure 1.** Arrhenius equation fits for degradation rates  $k$  (d<sup>-1</sup>) as a function of temperature (°C). These  
318 are based on the reported (full circles) and the estimated (empty circles) empirical values from literature.  
319 Lines are the best prediction and the shaded band is the 95% confidence interval of the prediction.



**Figure 2.** Estimated in-sewer removal (transformation) rates from excretion point to WWTP influent (in-sewer residence time = 4.5 h) for selected drug biomarkers, calculated using the identified Arrhenius regression equations. Error bars represent 95% confidence interval following Monte Carlo simulation. Asterisks (\*) indicates that the temperature is out of applicability range.

325 **Table 1.** Overview of selected biomarker stability studies from published literature.

No.	Reference	Chemical	Data source for extraction of <i>k</i>	Temp. (°C)	pH	DO (mg L <sup>-1</sup> )	Duration of experiment (h)	No. of samples taken	C <sub>0</sub> (µg L <sup>-1</sup> )	TSS (g L <sup>-1</sup> )
1	(Baker and Kasprzyk-Hordern, 2011)	COC, CE, 6MAM, NorCOC	Table	2, 19	7.4	-	72	4	1.0	-
2	(van Nuijs et al., 2012)	COC, EME, 6MAM	Graph	20	7.5	-	26	13	0.06–0.60	-
3	(Chen et al., 2013)	6MAM	Graph	4	7.4	-	336	6	>0.1	-
4	(Bisceglia and Lippa, 2014)	COC, EME, CE, NorCOC	Values reported	9, 23, 31	7.4	-	26	16	1.5–3.0	-
5	(Senta et al., 2014)	COC, 6MAM	Graph, values reported	20	7.5	-	72	7	0.2	-
6	(Thai et al., 2014)	COC, 6MAM	Values reported	20	7.5	-	12	9	10	-
7	(Mardal et al., 2016)	COC, EME, CE	Graph, Table	23	7-8	-	24	9	0.5-100	-
8	(Ramin et al., 2016)	COC, EME, CE, 6MAM	Values reported	14	8.6–8.8	10	48	9	10	0.32 ± 0.04
9	(McCall et al., 2016)	COC, CE, 6MAM, NorCOC	Values reported	21	8.0–8.9	5–8	24	11	2.0–3.0	0.14–0.29
10	(Devault et al., 2017)	COC, 6MAM	Values reported	20, 30	6.6, 7.6	-	24	7	1.0–3.0	-

<sup>1</sup>Used silanized amber glass bottles stored in the dark.

<sup>2</sup>Stability test performed in silanized glass flasks which were hand-shaken app. 10 times per hour.

<sup>3</sup>Bottles at 20°C were placed under fume cupboard uncapped and gently stirred 3 times per day (distilled water was used to compensate for evaporation). Bottle at 4°C was stored with cap on.

<sup>4</sup>Used Erlenmeyer flask equipped with foam stopper to allow air transfer. Reactor was shaken at 180 rpm in the dark.

<sup>5</sup>Glass bottles were capped with cotton plugs and placed in a thermostated cabinet.

<sup>6</sup>Used gravity sewer reactor with continuous mixing with magnetic stirrer (250 rpm) to enhance surface aeration.

<sup>7</sup>Urinary samples collected at a music festival was diluted with wastewater and incubated in a temperature water bath

<sup>8</sup>Transformation study was performed in a covered jacketed reactor equipped with an agitator and oxygen diffuser.

<sup>9</sup>Transformation study was conducted in Erlenmeyer flask on a shaker table in the dark. Autoclaved wastewater was chosen to represent abiotic transformation.

<sup>10</sup>Glass bottles were placed in the dark and aerobic conditions was maintained by shaking with a magnetic stir bar.

327 **Table 2.** Estimated  $k_{T25}$  (d<sup>-1</sup>) and  $\theta$  and their correlation for the selected drugs. Parameters are estimated  
 328 as the best fitted value together with 95% confidence interval. The predictions are valid in the reported  
 329 temperature range.

	$k_{T25}$ (d <sup>-1</sup> )	$\theta$	Correlation ( $k_{T25}$ and $\theta$ )	Temperature range (°C)
<b>COC</b>	1.48 (1.23, 1.75)	1.07 (1.04, 1.11)	0.06	9–31
<b>EME</b>	1.78 (1.03, 2.54)	1.18 (1.09, 1.28)	-0.75	9–31
<b>CE</b>	0.73 (0.61, 0.85)	1.10 (1.06, 1.13)	0.07	2–31
<b>6MAM</b>	0.64 (0.49, 0.78)	1.04 (1.00, 1.07)	0.49	2–30
<b>NorCOC</b>	1.44 (0.32, 2.57)	1.04 (0.90, 1.18)	0.29	9–31

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